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RESEARCH ARTICLE

Several natural products isolated from a red alga *Gracilaria lemaneiformis* and its evaluation of antialgal activity against six common red tide microalgae

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Abstract

The ethanol extracts of *Gracilaria lemaneiformis* that have inhibitory effects on *Karenia mikimotoi* and *Skeletonema costatum* were separated by liquid-liquid extraction using different polar solvents into five fractions with antialgal activities (petroleum ether, chloroform, ethyl acetate, *n*-butanol, and water-soluble fractions). These fractions were chromatographed on silica gel to give, after repeated preparative thin-layer chromatography (PTLC) purification processes, 1-β-D-ribofuranosyluracil (**1**), 3-hydroxymethyl-pyrrolopiperazine-2,5-dione (**2**), benzene-1,2-propanoic acid (**3**), 1-*O*-palmitoyl-2-*O*-palmitoleoyl-3-*O*-β-D-galactopyranosyl glycerol (**4**), 7-oxabicyclo[4.1.0]-heptan-3-ol (**5**), linoleic acid (**6**), 3,4-dimethoxy-6-(methoxymethyl)-tetrahydro-2H-pyran-2,5-diol (**7**), and 3,7,11,16-tetramethyl -2-heptadecen-1-ol (**8**). Five of them, natural products **1**, **2**, **5**, **7**, and **8**, were isolated from *Gracilaria lemaneiformis* for the first time, and three natural products (**3**, **5**, and **8**) were isolated from marine macroalgae for the first time. Among them, natural products (**1**, **2**, **3**, **4**, and **6**) showed the most obvious inhibition activities to the growth of *Karenia mikimotoi* and *Skeletonema costatum* at the concentration of 80 μg/mL. Therefore, antialgal activities of these five natural products against *Amphidinium carterae*, *Heterosigma akashiwo*, *Karenia mikimotoi*, *Phaeocystis globosa*, *Prorocentrum donghaiense*, and *Skeletonema costatum* were further tested at different concentrations (0.4, 2, 10, and 50 μg/mL). This was the first report of antialgal activities of five natural products (**1**, **2**, **3**, **4**, and **6**) to these six red tide microalgae. They showed significantly selective antialgal activities against all tested red tide microalgae. At the concentration of 50 μg/mL, the growth of *Amphidinium carterae*, *Heterosigma akashiwo*, *Karenia mikimotoi*, and *Phaeocystis globosa* was obviously inhibited; for *Karenia mikimotoi*, natural products **1**, **2**, and **6** have significant antialgal activities; the growth inhibition of *Skeletonema costatum* that was exposed to natural products **1**, **3**, and **4** was remarkable. Furthermore, by analyzing and comparing $EC_{50–96\ h}$ values, it has been determined that natural product **3** (natural product **4**) showed the superior application potential than potassium dichromate and some reported natural products (such as gossonorol isolated from *Porphyra yezoensis*, trehalose purified from *Ulva pertusa*) as a characteristic antialgal agent against *Amphidinium carterae* (*Phaeocystis globosa*). In addition, natural products **1** and **3** also showed good superiority than some reported natural products in inhibiting *Skeletonema costatum*; however, it was a pity that they were inferior to potassium dichromate in the inhibiting this red tide microalgae. Taken together, it is not hard to conclude that *Gracilaria lemaneiformis* was a good source of natural products with antialgal activities against some red tide microalgae.

Keywords Antialgal activity evaluation · EC_{50} · *Gracilaria lemaneiformis* · isolation · natural products · purification · red tide microalgae

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Introduction

Inhibitory effects of marine macroalgae on the growth of marine microalgae have been studied for more than 60 years (Alamsjah et al. 2005; Anderson et al. 1996; Fltcher 1975; Lee and Olsen 1985; Marshall and Orr 1949; McLachlan and Craigie 1964; Sun et al. 2018a, b, c), and have been fruitful especially in the past 30 years. The inhibition activities of some marine macroalgae against red tide microalgae, such as *Alexandrium tamarense* (Wang et al. 2007), *Chattonella marina* (Tang and Gobler 2011), *Cochlodinium polykrikoides* (Chowdhury et al. 2014; Jeong et al. 2000; Oh et al. 2010; Xu et al. 2005a, b), *Heterosigma akashiwo* (Alamsjah et al. 2005; Chowdhury et al. 2014; Hirao et al. 2012; Sun et al. 2016; Wang et al. 2009), *Karenia mikimotoi* (Nagayama et al. 2003; Sun et al. 2017, 2018a, b, c), *Ostreopsis* cf. *ovata* (Accoroni et al. 2015), *Prorocentrum minimum* (Li et al. 2007; Tang and Gobler 2011), *Skeletonema costatum* (An et al. 2008; Bie et al. 2011; Cui 2014; Gao et al. 2018; Jin 2005; Lu 2011), and other marine microalgae (Accoroni et al. 2015; Alamsjah et al. 2005; Ben Gharbia et al. 2017; Cai 2016; Cho et al. 1999; Konig et al. 1999; Manilal et al. 2010; El Hattab et al. 2015; Takedai et al. 2003; Tang and Gobler 2011; Tian 2009; Wang et al. 2007, 2012a, b; Wu 2016; Ye and Zhang 2013; Yu et al. 2010), have been reported. In our previous work, researches between marine macroalgae and red tide microalgae were analyzed using CiteSpace, and it was found that allelopathic interaction between them has been developed into an active research area during the last 20 years (Sun et al. 2019). Further, based on these reports have been published in the Web of Science, Springer, Google Scholar, and CNKI between 1990 and 2019, we also found that more than 120 species of marine macroalgae have antialgal activities against red tide microalgae (Fig. 1a), and red macroalgae in Rhodophyta have highest proportion among them. According to different orders of these red macroalgae, we summarize their classification and found that orders Gracilariales and Gigartinales have the most species with antialgal activities (Fig. 1b).

Gracilaria lemaneiformis, a red macroalgae belong to order Gracilariales, occurs naturally in coastal areas of Shandong Peninsula in northern China. Researchers have found a lot of solvent extracts from *Gracilaria lemaneiformis* have antimutagenic (Chen et al. 2005; Zhang et al. 2005), antitumor (Mei et al. 2006; Zhang et al. 2005), antioxidant (Chen et al. 2005, 2008), antialgal (Lu 2011; Sun et al. 2011), and other biological activities (Chen et al. 2007, 2008). In fact, antialgal effects of *Gracilaria lemaneiformis* on growth of *Prorocentrum donghaiense* and *Alexandrium tamarense* in co-culture were studied in early time (Wang et al. 2006). Also, Liu (2006) reported that antialgal interaction between *Gracilaria lemaneiformis* and three red tide microalgae (*Chaetoceros curvisetus*, *Skeletonema costatum* and *Scrippsiella trochoidea*). Shao et al. (2011) and Lei et al. (2010) showed the inhibitory effects of *Gracilaria lemaneiformis* on the growth of *Heterosigma akashiwo* and *Prorocentrum micans*. Methanol and aqueous extracts of *Gracilaria lemaneiformis* were found to inhibit the growth of *Karenia mikimotoi* and *Skeletonema costatum* (Sun et al. 2011). However, a few antialgal substances from *Gracilaria lemaneiformis* were structurally elucidated (Lu 2011; Sun et al. 2017). Lu (2011) reported that linoleic acid isolated from *Gracilaria lemaneiformis* was inhibitory allelochemical against *Skeletonema costatum*. Our group has already isolated gossonorol and other six compounds from *Gracilaria lemaneiformis* (Sun et al. 2017), for the interpretation of antialgal activities of methanol extracts of this marine macroalgae (Sun et al. 2011, 2012).

A previous experiment provided evidence for inhibitory effects of different solvent extracts (petroleum ether, chloroform, and ethyl acetate) from *Gracilaria lemaneiformis* on *Karenia mikimotoi* (Sun et al. 2012), but the nature of the

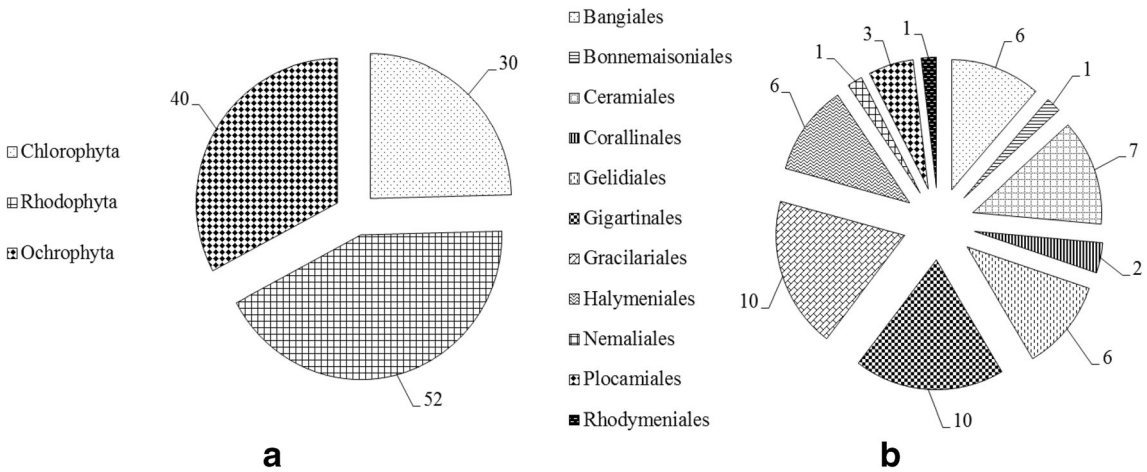

Fig. 1 Distribution in different phyla (**a**) and orders (**b**) of marine macroalgae with antialgal activity. Data extracted from related studies since 1960, and the number in a pie chart represents the total number of marine macroalgae species belong to this phylum or order.

antialgal active compound(s) was not investigated. Different extraction and isolation methods are likely to get different results; therefore, we were likely to find some compounds with antialgal activities that were different from those reported. Hopefully, present paper purified eight natural products, and six of them have not been characterized before. Based on that, this work was to evaluate their antialgal activities against six species of red tide microalgae (*Amphidinium carterae*, *Heterosigma akashiwo*, *Karenia mikimotoi*, *Phaeocystis globosa*, *Prorocentrum donghaiense*, and *Skeletonema costatum*) and obtain important parameters, e.g., $EC_{50–96\ h}$ values for future practical HAB control. *Gracilaria lemaneiformis* was, assuredly, a rich source of natural products with antialgal activities. It can be used to develop antialgal inhibitor against red tide microalgae in future research.

Materials and methods

HAB algae and macroalgae

Amphidinium carterae, *Heterosigma akashiwo*, *Karenia mikimotoi*, *Phaeocystis globosa*, *Prorocentrum donghaiense*, and *Skeletonema costatum* were cultured aseptically in Guillard's f/2 medium (Guillard and Ryther 1962), 20 °C, and 60 μmol photons/m^2/s using fluorescent lamps with a 16/8 dark/light cycle.

Gracilaria lemaneiformis collected in July 2017 from the coast of Fujian, China, was purchased from a wholesaler, identified by Prof. Binlun Yan (Jiangsu Key Laboratory of Marine Biotechnology, Jiangsu Ocean University), and washed with a brush to carefully remove attached organisms. And then, these materials were cut into small pieces (ca. 2.0 cm of length) and freeze-dried. After freeze-drying treatment, these pieces were ground to make powder using a blender for 1 min.

Extraction, isolation, purification, and identification

The milled sample (500 g) was extracted twice with 100% ethanol (2.5 L, 36 h, and 1.5 L, 12 h). The extracts were concentrated to 300 mL under reduced pressure in an evaporator and filtered through a Whatman GF/C filter. The filtrates were added to H_2O (400 mL), stayed overnight in the refrigerator at 4 °C, and centrifuged for 10 min at 7000×*g* under low temperature. The supernatants were freeze-dried, and then, the alcoholic extracts of *Gracilaria lemaneiformis* (AEGL, 58.775 g) were obtained. The extracts (AEGL) were further isolated by the following methods (Fig. 2).

Brown mixed solution could be obtained by the leaching process of the extracts with a two-liquid phase solvent system (400 mL of petroleum ether/H_2O (1:3, *v*:*v*)) at room temperature (20 °C) for 24 h in the darkness under agitation with mill speed of 30 r/min. The above leaching process was repeated three times. Extraction liquid was combined and filtered. And then, brown mixed solution was transferred to a 2000-mL separation funnel, shaken for 5 min, and allowed to stand for 30 min. Aqueous phase and petroleum ether phase were got, respectively. Petroleum ether phase was kept. Aqueous phase was evaporated to remove petroleum ether and pretreated using liquid-liquid extraction based on chloroform. This extraction was repeated three times. The resultant mixture was transferred to a 2000-mL separation funnel, shaken for 5 min, and allowed to stand for 30 min. Chloroform phase was retained, and upper layer was evaporated to remove chloroform to obtain aqueous phase. Then, 100 mL of ethyl acetate was added, and aqueous phase was extracted three times with ethyl acetate. Ethyl acetate phase was retained. The raffinate was evaporated to remove ethyl acetate, and 100 mL of *n*-butanol was added. This was followed by separation using a separation funnel and repeated three times. *N*-butanol phase and bottom layer were collected, respectively. In this way, five fractions (4.072 g petroleum ether fraction, 0.589 g chloroform fraction, 0.689 g ethyl acetate fraction, 9.85 g *n*-butanol fraction, and 16.58 g water-soluble fraction) were separated from the alcoholic extracts. All of the fractions were collected separately, evaporated to dryness, weighed, redissolved with ethanol (10 mg/mL approximately), and filtered through a 0.22-micron syringe filter as a stock solution before bioassays.

Isolation and purification of five fractions mentioned above were performed as follows. First, five fractions were applied to silica gel (200–300 mesh) column (3.0 × 100 cm) (mobile phase: chloroform/acetone/formic acid = 18:1:1 (*v*:*v*:*v*) and chloroform/methanol = 16:1 (*v*:*v*), alternatively), respectively. Ten microliter of sample was injected onto the top of the column and then eluted with eluent at a flow rate of 2.0 mL/min. The collection of the 10-mL sub-fraction was started immediately after the addition of eluent. Antialgal activities of sub-fractions were determined by a bioassay using *Karenia mikimotoi* and *Skeletonema costatum*, and then, they were pooled and concentrated, respectively. Secondly, sub-fractions with antialgal activities were respectively loaded on silica gel (100–200 mesh) column (1.5 × 50 cm) and washed with chloroform/acetone/formic acid (15:3:2, *v*:*v*:*v*) at a flow-rate of 1.0 mL/min. Collection of the 5-mL elution component was started immediately. Further, in turn, the active elution components were subjected to preparative TLC on silica gel G plates (500 μm, Merck) and collected according to R_f value under UV light (UV 254 nm). PTLC was repeated two times and carried out in repetitive way. Finally, active components were purified by Sephadex LH-20 column (1.0 × 30 cm) (mobile phase: methanol), respectively. Systemic solvent separation, two phase solvent extraction, silica gel column chromatography, PTLC and Sephadex LH-20 column

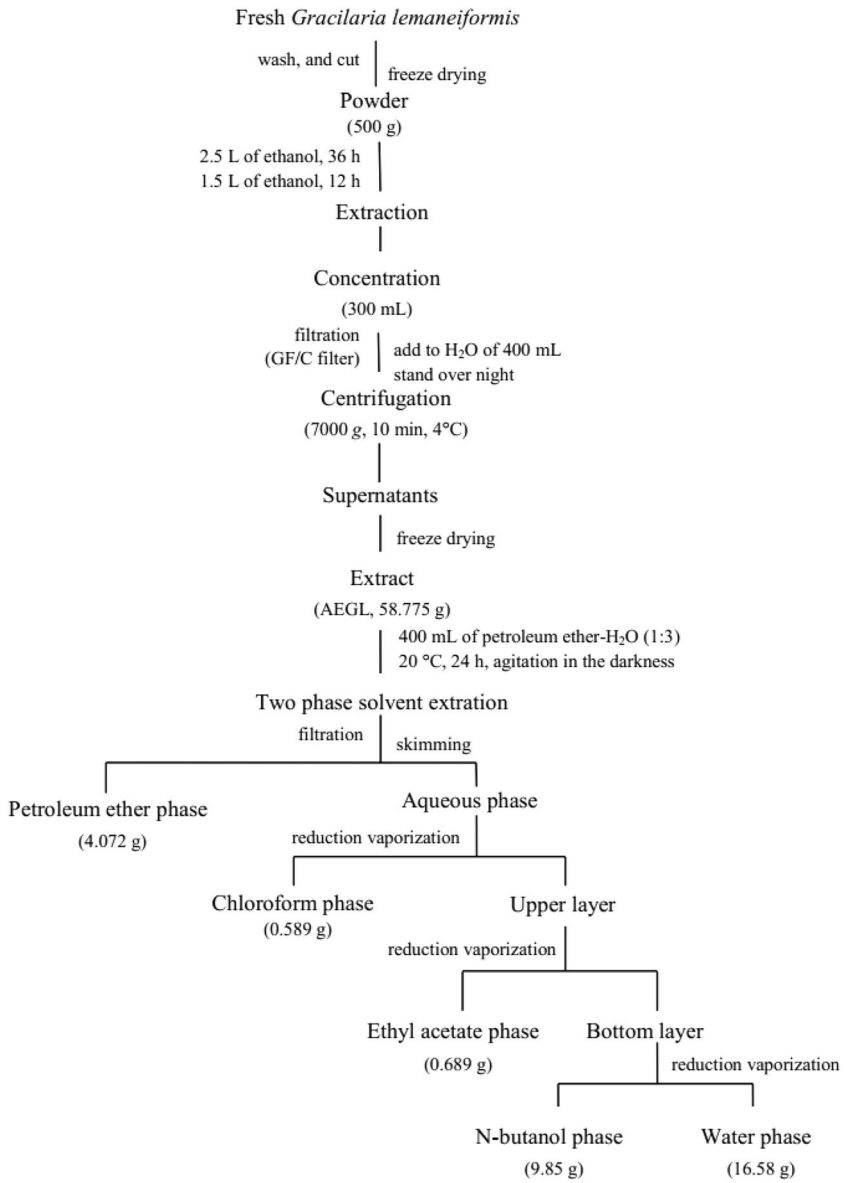

Fig. 2 A flow chart of extraction and isolation

chromatography methods were applied to eight natural products. Finally, each natural product was dissolved in ethanol at a concentration of 5 mg/mL, filtered through a 0.22-micron syringe filter, and assessed antialgal activity to tested red tide microalgae. At the same time, the structural identification of these natural products was carried out by comparison of HR-ESI-MS, ^{1}H-NMR, and ^{13}C-NMR with spectral data.

Growth assays

Crude extracts and multiple isolated constituents were monitored by a bioassay using *Karenia mikimotoi* and *Skeletonema costatum*. Natural seawater was obtained from the coast of Lianyungang, China, collected in precleaned polyethylene tanks and aged. And then aged natural seawater was filtered through an acid-cleaned 0.22 μm Millipore membrane filter.

Growth assays of AEGL

The growth inhibition of AEGL was evaluated by algal bioassays with *Karenia mikimotoi* and *Skeletonema costatum* on the method reported by Sun et al. (2016) with some modifications. One hundred microliters of the solvent-partitioned fraction with different concentrations was added to Erlenmeyer flasks containing 5 mL of algal inoculant and 44.9 mL of culture medium (initial concentration of fraction in suspensions of two microalgae: 400, 800, 1200 μg/mL). Controls received the same volume of ethanol. The initial cell numbers were set at 9×10^4 cells/mL for *Karenia mikimotoi* and 21×10^4 cells/mL for *Skeletonema costatum*. The tested red tide microalgae were cultured under 60 μmol photons/m^2/s (light-dark = 16–8 h) at 20 °C for 10 days. There were four replicates for every treatment used in this experiment.

Growth assays of isolated constituents in the process of isolation and purification

Ten microliters of each isolated constituent was added to teat glass containing 0.5 mL of algal inoculant and 4.49 mL of culture medium (initial concentration of isolated constituent in suspensions of tested red tide microalgae 80 μg/mL); their growth effects on tested red tide microalgae (*Karenia mikimotoi* and *Skeletonema costatum*) were measured on the method reported by Sun et al. (2016) with some modifications. Controls received the same volume of ethanol. The initial cell numbers were set at 22 × 10^4 cells/mL for *Karenia mikimotoi* and 26 × 10^4 cells/mL for *Skeletonema costatum*. There were four replicates for every treatment used in this experiment, and the culture conditions were the same as mentioned above. This experiment lasted for 4 days.

The evaluation of antialgal activities of five natural products

Five natural products with antialgal activities were obtained from our research. Antialgal ability of each natural product (0.4, 2, 10, and 50 μg/mL) against six red tide microalgae (*Amphidinium carterae*, *Heterosigma akashiwo*, *Karenia mikimotoi*, *Phaeocystis globosa*, *Prorocentrum donghaiense*, and *Skeletonema costatum*) was measured. Potassium dichromate was used as a positive control. The culture conditions were conducted as described above. After 4-day culture, the cells were counted. On day 4, the growth inhibition of each natural product against tested red tide microalgae was determined according to the method mentioned in our previous work (Sun et al. 2012). $EC_{50–96\,h}$ was calculated as the method reported by Marklund and Marklun (1974).

Data process and statistical analysis

Cell numbers of red tide microalgae were counted by hemocytometer. All the data of the growth assays in this study were analyzed by ANOVA and Tukey's test.

Results

Solvent fractions of AEGL from *Gracilaria lemaneiformis*

AEGL (alcoholic extracts of *Gracilaria lemaneiformis*) showed significant ($P < 0.05$) inhibitory activities against the growth of *Karenia mikimotoi* and *Skeletonema costatum*, and the inhibitory effects to these two red tide microalgae were above 50% at the concentration of 800 μg/mL on day 10 (Fig. 3). Further, five fractions are gained in AEGL by using systematical solvent separation method. Among all these fractions, the dark brown water-soluble fraction (WS, 16.58%) showed the highest yield followed by deep yellow *n*-butanol fraction (BN, 9.85%), invisible green petroleum ether fraction (PE, 6.93%), the yellowy ethyl acetate fraction (EA, 0.689%), and the reseda chloroform fraction (CF, 0.589%). Their inhibitory effects on the growth of *Karenia mikimotoi* and *Skeletonema costatum* are shown in Table 1. Results exhibited that they restrained these two tested red tide microalgae and inhibitory activities of BN and WS against *Karenia mikimotoi* and *Skeletonema costatum* were stronger than those of other three fractions. Then, isolation and purification of the above-mentioned five fractions were conducted.

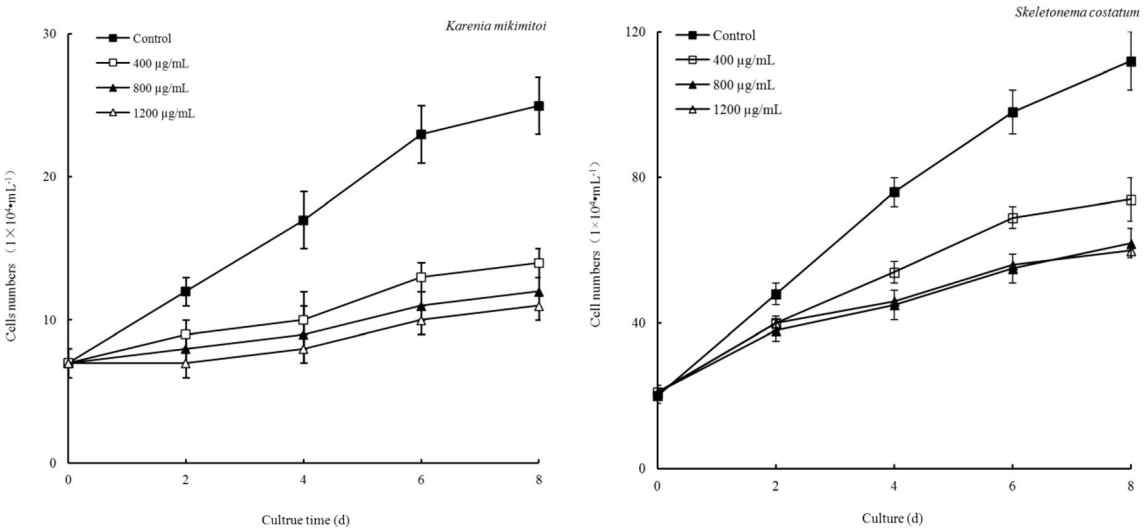

Fig. 3 Effect of the ethanol extracts of *Gracilaria lemaneiformis* on the growth of *Karenia mikimotoi* and *Skeletonema costatum*

Table 1 Antialgal activities of five solvent fractions (80 μg/mL) from ethanol extracts of *Gracilaria lemaneiformis* against the growth of *Karenia mikimotoi* and *Skeletonema costatum*

Test red tide microalgae	PE	CF	EA	BN	WS
Karenia mikimotoi	+	+	++	++	++
Skeletonema costatum	+	+	+	++	++

− no antialgal activity, + weak antialgal activity (20–50%), ++ strong antialgal activity (over 50%), *PE* petroleum ether fraction, *CF* chloroform fraction, *EA* ethyl acetate fraction, *BN* *n*-butanol fraction, *WS* water-soluble fraction

Isolation, purification, and identification

PE (3.0 g) and CF (0.4 g) were respectively eluted through silica gel column chromatography with mixtures of chloroform/acetone/formic acid (18:1:1, *v*:*v*:*v*) and further purified by PTLC (twice, mobile phase: chloroform/acetone/formic acid (15:3:2, *v*:*v*:*v*)) to yield three samples with antialgal activities: FPE_{11} (0.232 g), FCF_{11} (0.024 g), and FCF_{21} (0.025 g) (Table 2).

EA (0.4 g), BN (5 g), and WS (5 g) were respectively subjected to silica gel column chromatography using chloroform/methanol (16:1, *v*:*v*) as eluent and PTLC (twice, mobile phase: chloroform/acetone/formic acid (15:3:2, *v*:*v*:*v*) to yield five samples with antialgal activities: FEA_{21} (0.012 g), FEA_{31} (0.017 g), FEA_{32} (0.062 g), FBN_{11} (0.713 g), and FWS_{11} (0.384 g) (Table 2).

All eight samples showed antialgal activities against *Karenia mikimotoi* and *Skeletonema costatum*. The difference was as follows: FPE_{11}, FCF_{11}, FCF_{21}, FEA_{21}, and FEA_{32} significantly inhibited the growth of these two test red tide microalgae, and other three samples only have weak antialgal activities against *Karenia mikimotoi* and *Skeletonema costatum* (Table 2). Therefore, these eight samples were finally purified through Sephadex LH-20 column chromatography to obtain eight natural products with antialgal activities, namely FPE_{111} (0.218 g, yield 0.077%, dw basis of AEGL), FCF_{111} (0.017 g, yield 0.043%), FCF_{211} (0.018 g, yield 0.063%), FEA_{211} (0.009 g, yield 0.037%), FEA_{311} (0.009 g, yield 0.015%), FEA_{321} (0.051 g, yield 0.105%), FBN_{111} (0.685 g, yield 2.165%), and FWS_{111} (0.363 g, yield 2.014%) (Table 2).

On the basis of the above experiments, the structures of eight natural products were identified. FPE_{111}, FCF_{111}, FCF_{211}, FEA_{211}, FEA_{311}, FEA_{321}, FBN_{111}, and FWS_{111} were as follows: 1-β-D-ribofuranosyluracil (**1**), 3-hydroxymethyl-pyrrolopiperazine-2,5-dione (**2**), benzene-1,2-propanoic acid (**3**), 1-*O*-Palmitoyl-2-*O*-palmitoleoyl-3-*O*-β-D-galactopyranosyl glycerol (**4**), 7-Oxabicyclo[4.1.0]-heptan-3-ol (**5**), linoleic acid (**6**), 3,4-dimethoxy-6-(methoxymethyl)-tetrahydro-2H-pyran-2,5-diol (**7**), and 3,7,11,16-tetramethyl-2-heptadecen-1-ol (**8**). Their NMR spectroscopic data are listed in Tables 3, 4, 5, and 6. Due to these, eight natural products were known compounds, so the specific process of structural identification was omitted in this paper. And the structures of these natural products are shown in Fig. 4.

Overall, eight natural products with antialgal activities were isolated from *Gracilaria lemaneiformis*. And among them, five natural products **1**, **2**, **3**, **4**, and **6** have higher inhibitory activities against *Karenia mikimotoi* and *Skeletonema costatum* at the concentration of 80 μg/mL. So far, there was no study on inhibition activities of these five natural products against red tide microalgae. Thus, antialgal activities of natural products **1**, **2**, **3**, **4**, and **6** were tested against the common red tide microalgae *Amphidinium carterae*, *Heterosigma akashiwo*, *Karenia mikimotoi*, *Phaeocystis globosa*, *Prorocentrum donghaiense*, and *Skeletonema costatum* in this work.

Table 2 Yield of antialgal samples of the ethanol extracts from *Gracilaria lemaneiformis* during isolation and purification steps

Solvent fractions												
PE		CF			EA		BN		WS			
3.0 g		0.4 g			0.4 g		5.0 g		5.0 g			
Silica gel column chromatography (twice)												
	FPE_1^+	FCF_1^+	FCF_2^+	FCF_3^-	FEA_1^+	FEA_2^+	FEA_3^{++}	FBN_1^{++}	FBN_2^-	FBN_3^-	FBN_4^-	FWS_1^{++}
Yield (g)	1.663	0.187	0.098	–	0.084	0.072	0.085	1.424	–	–	–	1.255
PTLC (twice)												
	FPE_{11}^{++}	FCF_{11}^{++}	FCF_{21}^{++}	FEA_{11}^-	FEA_{21}^{++}	FEA_{22}^-	FEA_{31}^+	FEA_{32}^{++}	FEA_{33}^-	FBN_{11}^+	FWS_{11}^+	
Yield (g)	0.232	0.024	0.025	–	0.012	–	0.017	0.062	–	0.713	0.384	
Sephadex LH-20 column chromatography (once)												
	FPE_{111}^{++}	FCF_{111}^{++}	FCF_{211}^{++}	–	FEA_{211}^{++}	–	FEA_{311}^+	FEA_{321}^{++}	–	FBN_{111}^+	FWS_{111}^+	
Yield (g)	0.218	0.017	0.018		0.009		0.009	0.051		0.685	0.363	

"–" no antialgal activity, "+" weak antialgal activity (20–50%), "++" strong antialgal activity (over 50%)

Table 3 NMR spectroscopic data of natural products **1**, **2**, and **3** in $CDCl_3$

C. no.	Natural product **1**			Natural product **2**			Natural product **3**		
	δ^{13}C NMR	H	δ ^{1}H NMR	δ^{13}C NMR	H	δ ^{1}H NMR	δ^{13}C NMR	H	δ ^{1}H NMR
C-1	–	–	–	–	–	–	167.6 (C)	H-1	7.22 (m)
C-2	102.6 (C)	–	–	169.1 (C)	–	–	30.3 (CH_2)	H-2	7.66 (m)
C-3	–	–	–	59.7 (CH)	H-3	4.13 (d J = 4.8 Hz)	19.3 (CH_2)	H-3	7.66 (m)
C-4	166.2 (C)	–	–	–	–	–	–	H-4	7.22 (m)
C-5	152.5 (CH)	H-5	5.61 (d, J = 8.4 Hz)	164.7 (C)	–	–	–	–	–
C-6	142.7 (CH)	H-6	7.92 (d, J = 8.4 Hz)	58.2 (CH)	H-6	3.65 (m)	–	–	–
C-1' (C-7)	90.7 (CH)	H-1'	5.81 (d, J = 4.2 Hz)	44.9 (CH_2)	–	–	132.2 (C)	–	–
C-2' (C-8)	71.3 (CH)	–	–	21.5 (CH_2)	–	–	130.5 (CH)	–	–
C-3' (C-9)	75.7 (CH)	–	–	28.8 (CH_2)	–	–	128.7 (CH)	–	–
C-4' (C-10)	86.4 (CH)	–	–	–	H-10α H-10β	3.70 (m) 3.48 (m)	–	–	–

Antialgal activity evaluation of five natural products against six red tide microalgae

Amphidinium carterae, *Heterosigma akashiwo*, *Karenia mikimotoi*, *Phaeocystis globosa*, *Prorocentrum donghaiense*, and *Skeletonema costatum* were employed for the inhibition tests. In Figs. 5a, b, d, and e, inhibition effects of five natural products (**1**, **2**, **3**, **4**, and **6**) on *Amphidinium carterae*, *Heterosigma akashiwo*, *Phaeocystis globosa*, and *Prorocentrum donghaiense* grow more and more with their increasing concentration (0.4~50 μg/mL). There were significant ($P < 0.05$) differences between cell numbers of control groups and treated groups with added five natural products. At 50 μg/mL, all natural products for 50% inhibition of growth of these four red tide microalgae were found (Fig. 6b). However, among them, only natural products **1** and **2** significantly ($P < 0.05$) restrained *Karenia mikimotoi* (Fig. 5c) and growth inhibition of these two natural products to this red tide microalgae was 53.4% and 76.1%, respectively (Fig. 6b); in addition to natural products **2** and **6**, other three natural products strongly ($P < 0.05$) inhibited *Skeletonema costatum* (Figs. 5f and 6b). These five natural products have selective inhibitory activities against tested six red tide microalgae.

Antialgal ability of potassium dichromate against six species of red tide microalgae increased significantly ($P < 0.05$) with its increasing concentration (Fig. 6a). At 16 μg/mL, growth inhibition of potassium dichromate on *Amphidinium carterae*, *Heterosigma akashiwo*, *Karenia mikimotoi*, *Phaeocystis globosa*, *Prorocentrum donghaiense*, and *Skeletonema costatum* was 83.5%, 42.9%, 48.9%, 39.7%, 76.9%, and 95.0%, respectively.

Based on antialgal activity evaluation, $EC_{50–96\ h}$ values of five natural products abovementioned for six red tide microalgae were determined for the first time (Table 7). In OECD (1984), $EC_{50–96\ h}$ value of tested compound could directly indicate its toxicity to the test algae, namely, $EC_{50–96\ h}$ value was in the range < 1 μg/mL, 1~10 μg/mL, 10~100 μg/mL, and > 100 μg/mL; the corresponding toxicity was extremely high toxic, high toxic, medium toxicity, and low toxicity, respectively. And potassium dichromate was used as positive control in algae test standard according to the recommendation of this reference. Advantages of algal inhibition activities of potassium dichromate and five natural products were analyzed, by comparing their toxicity and $EC_{50–96\ h}$ values for each tested red tide microalgae, in order to select more potential compounds from them in further application. According to these standard mentioned above (OECD 1984), for *Amphidinium carterae*, potassium dichromate, natural products **2** and **3** exhibited high toxicities, and other three natural products showed medium toxicities. And $EC_{50–96\ h}$ value of natural product **3** for this microalgae was significant ($P < 0.05$) lower than that of potassium dichromate; $EC_{50–96\ h}$ values of five natural products for *Heterosigma akashiwo* and *Phaeocystis globosa* were significant ($P < 0.05$) less than those of potassium dichromate. Among them, other natural products showed high toxicities for *Heterosigma akashiwo* except natural product **1** and potassium dichromate, four natural products exhibited high toxicities for *Phaeocystis globosa* besides natural product 5 and potassium dichromate which only showed medium toxicity; natural products **2**, **4**, and **6** had high toxicities for *Karenia mikimotoi*, and their $EC_{50–96\ h}$ values were significant ($P < 0.05$) lower than those of potassium dichromate; natural product **2** and potassium dichromate for *Prorocentrum donghaiense*, natural products **1**, **3**, **4**, and potassium dichromate for *Skeletonema*

Table 4 NMR spectroscopic data of natural product **4** in $CDCl_3$

C. no.	Natural product **4**						
	δ^{13}C NMR	H	δ ^{1}H NMR	δ^{13}C NMR	δ^{13}C NMR	H	δ ^{1}H NMR
C-1	62.9 (CH_2)	H-1α H-1β	4.39 (dd, J = 12.0, 3.0 Hz) 4.21 (dd, J = 12.0, 6.6 Hz)	C-4" ~C-7"	29.1~30.0 (CH_2)	–	–
C-2	70.2 (CH)	H-2	5.34 (m)	C-8"	27.2 (CH)	–	–
C-3	69.1(CH_2)	H-3α H-3β	3.89 (dd, J = 13.8, 5.4) 3.71 (dd, J = 12.0, 6.6)	C-9"	129.7(CH)	H-9"	5.34 (m)
C-1'	173.9 (C)	–	–	C-10"	130.0(CH_2)	H-10"	5.34 (m)
C-2'	34.3 (CH_2)	–	–	C-11"	27.2 (CH_2)	–	–
C-3'	24.9 (CH_2)	–	–	C-12" ~ C-15"	29.1~30.0 (CH_2)	–	–
		–	–	C-16"	–	H-16"	0.88 (t, J = 7.2 Hz)
C-4'~ C-13'	29.1~30.0 (CH_2)	–	–	C-18"	14.1(CH_3)	–	–
C-14'	31.9 (CH_2)	–	–	C-1"'	104.1 (CH)	H-1"'	4.26 (d, J = 7.2 Hz)
C-15'	22.7 (CH_2)	–	–	C-2"'	71.3 (CH)	–	–
C-16'	14.1 (CH_3)	H-16'	0.89 (t, J = 7.0 Hz)	C-3"'	69.1 (CH)	–	–
C-1"	173.5 (C)	–	–	C-4"'	74.7 (CH)	–	–
C-2"	4.2 (CH_2)	–	–	C-5"'	73.5 (CH)	–	–
C-3"	24.9 (CH_2)	–	–	C-6"'	61.9 (CH_2)	–	–

costatum were high toxic; and other natural products for these two microalgae were medium or low toxic. It was a pity that $EC_{50–96\ h}$ values of these five natural products for *Prorocentrum donghaiense* and *Skeletonema costatum* were significant ($P < 0.05$) higher than those of potassium dichromate. Based on the above analysis, we obtained several natural products with demonstrably antialgal superior than potassium dichromate against *Amphidinium carterae*, *Heterosigma akashiwo*, *Karenia mikimotoi*, and *Phaeocystis globosa*. These results were as follows: natural product **3** to *Amphidinium carterae*; natural products **1**, **2**, **3, 4**, and **6** for *Heterosigma akashiwo* and *Phaeocystis globosa*; and natural products **2**, **4**, and **6** against *Karenia mikimotoi*.

Table 5 NMR spectroscopic data of natural products **5** and **6** in $CDCl_3$

C. no.	Natural product **5**				Natural product **6**		
	δ^{13}C NMR	H	δ ^{1}H NMR		δ^{13}C NMR	H	δ ^{1}H NMR
C-1	35.6 (C)	–	–	C-1	178.8 (C)	–	–
C-2	47.6 (CH_2)	–	–	C-2	33.8 (CH_2)	–	–
C-3	63.5 (CH)	H-3	3.75 (m)	C-3	24.7 (CH_2)	–	–
C-4	41.5 (CH_2)	–	–	C-4~C-7	29.0~29.6 (CH_2)	–	–
C-5	67.8 (C)	–	–	C-8	27.2 (CH_2)	–	–
C-6	70.0 (C)	–	–	C-9	127.9 (CH)	H-9	5.39 (m)
C-7	34.2 (CH_2)	–	–	C-10	130.2 (CH)	H-10	5.39 (m)
C-8	128.5 (CH)	H-8	5.14 (d, J = 14.4 Hz)	C-11	25.6 (CH_2)	–	5.39 (m)
C-9	129.3 (CH)	H-9	5.36 (m)	C-12	130.0 (CH)	H-12	5.39 (m)
C-10	14.7 (CH_3)	H-10	1.68 (d, J = 7.2 Hz)	C-13	128.1 (CH)	H-13	3.70 (m)
C-11	25.3 (CH_3)	H-11	0.92 (s)	C-14	27.2 (CH_2)	–	–
C-12	29.6 (CH_3)	H-12	1.17 (s)	C-15	29.7 (CH_2)	–	–
C-13	20.1 (CH_3)	H-13	1.14 (s)	C-16	31.5 (CH_2)	–	–
C-14				C-17	22.6 (CH_2)	–	–
				C-18	14.1 (CH_3)	H-18	0.92 (t, J = 7.2 Hz)

Table 6 NMR spectroscopic data of natural products **7** and **8** in $CDCl_3$

C. no.	Natural product **7**				Natural product **8**		
	δ^{13}C NMR	H	δ ^{1}H NMR		δ^{13}C NMR	H	δ ^{1}H NMR
C-1	92.4 (CH_2)	–	–	C-1	59.2 (CH_2)	H-1	4.09 (m)
C-2	78.4 (CH)	H-2	3.49 (s)	C-2	125.9 (CH)	H-2	5.38 (m)
C-3	82.5 (CH)	H-3	3.54 (s)	C-3	137.4 (CH)	–	–
C-4	67.7 (CH)	–	–	C-4	40.1 (CH_2)	–	–
C-5	73.1 (CH)	–	–	C-5	25.9 (CH_2)	–	–
C-6	73.7 (CH)	H-6	3.32 (s)	C-6	38.1 (CH_2)	–	–
C-7	58.7 (CH_3)	–	–	C-7	33.4 (CH)	–	–
C-8	57.4 (CH_3)	–	–	C-8	37.9 (CH_2)	–	–
C-9	58.9 (CH_3)	–	–	C-9	25.5 (CH_2)	–	–
				C-10	37.3 (CH_2)	–	–
				C-11	33.5 (CH)	–	–
				C-12	38.1 (CH_2)	–	–
				C-13	25.1 (CH_2)	–	–
				C-14	40.7 (CH_2)	–	–
				C-15	28.6 (CH)	–	–
				C-16	27.4 (CH_2)	–	–
				C-17	23.0 (CH_3)	H-17	0.90 (d, J = 4.8 Hz)
				C-18	22.9 (CH_3)	H-18	0.90 (d, J = 4.8 Hz)
				C-19	20.1(CH_3)	H-19	0.90 (d, J = 4.8 Hz)
				C-20	20.1(CH_3)	H-20	0.90 (d, J = 4.8 Hz)
				C-21	16.1(CH_3)	H-21	1.64 (s)

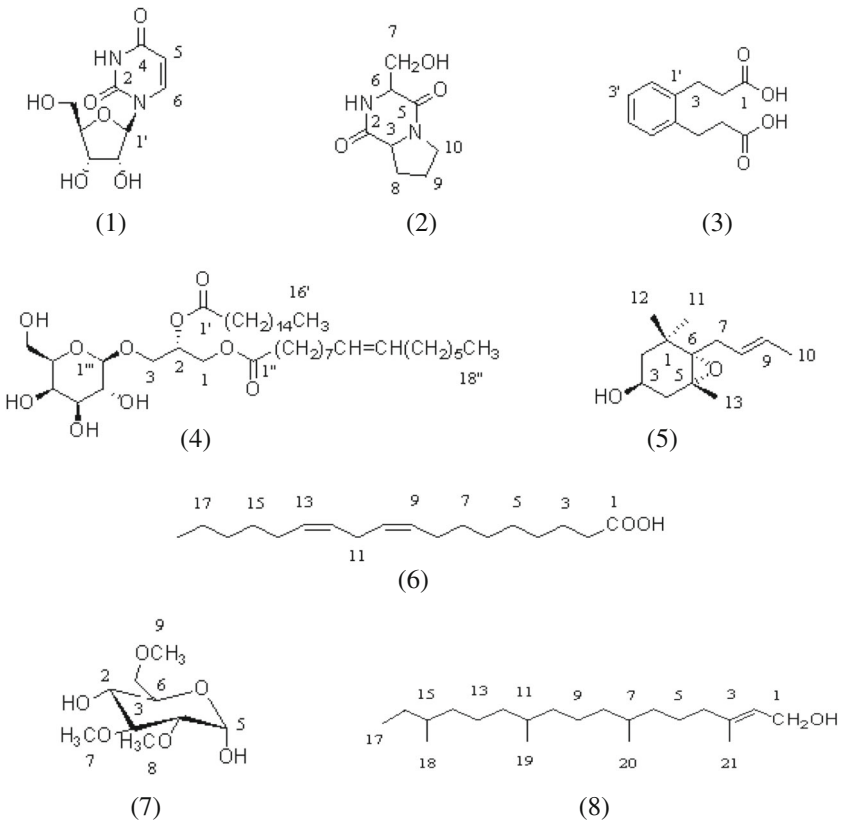

Fig. 4 Structure of eight natural products isolated from *Gracilaria lemaneiformis*

Discussions

Some red seaweeds which have shown notable inhibitory or algicidal activities to red tide microalgae that can prevent the development of microalgae or even kill them (Alamsjah et al. 2005; An et al. 2008; Jeong et al. 2000; Luyen et al. 2009; Suzuki et al. 1998; Tanabe et al. 1993; Xu 2008; Xu 2005; Xu et al. 2005a, b). For example, lectin which prepared from *Gracilaria verrucosa* was investigated for its activity against the growth of *Chattonella antiqua* and found that this toxic red tide microalgae was completely suppressed at 50 μg/mL (Tanabe et al. 1993). Suzuki et al. (1998) reported that ethanol

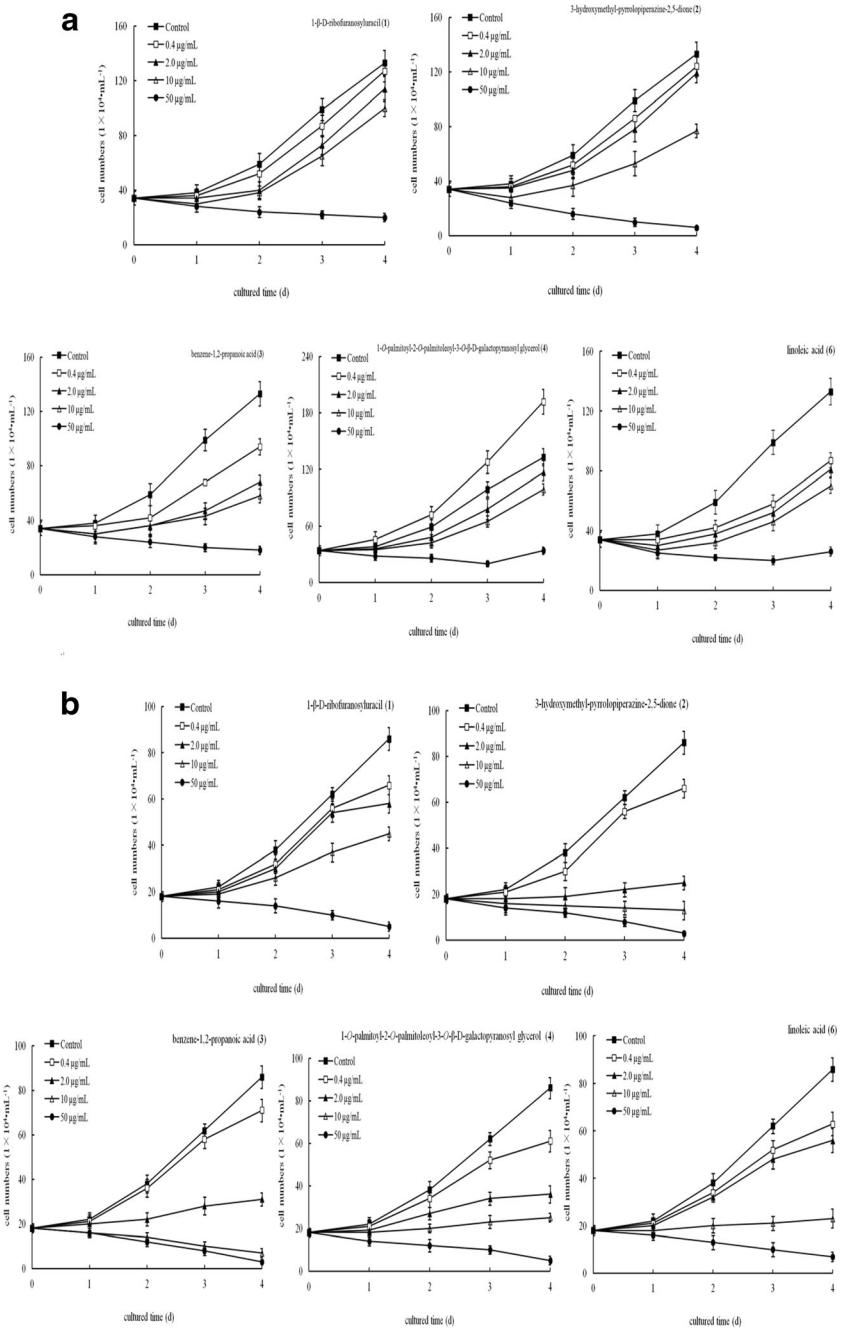

Fig. 5 (**a**) Effects of natural products (**1**, **2**, **3**, **4**, and **6**) on the growth of *Amphidinium carterae*. (**b**) Effects of natural products (**1**, **2**, **3**, **4**, and **6**) on the growth of *Heterosigma akashiwo*. (**c**) Effects of natural products (**1**, **2**, **3**, **4**, and **6**) on the growth of *Karenia mikimotoi*. (**d**) Effects of natural products (**1**, **2**, **3**, **4**, and **6**) on the growth of *Prorocentrum donghaiense*. (**e**) Effects of natural products (**1**, **2**, **3**, **4**, and **6**) on the growth of *Phaeocystis globosa*. (**f**) Effects of natural products (**1**, **2**, **3**, **4**, and **6**) on the growth of *Skeletonema costatum*.

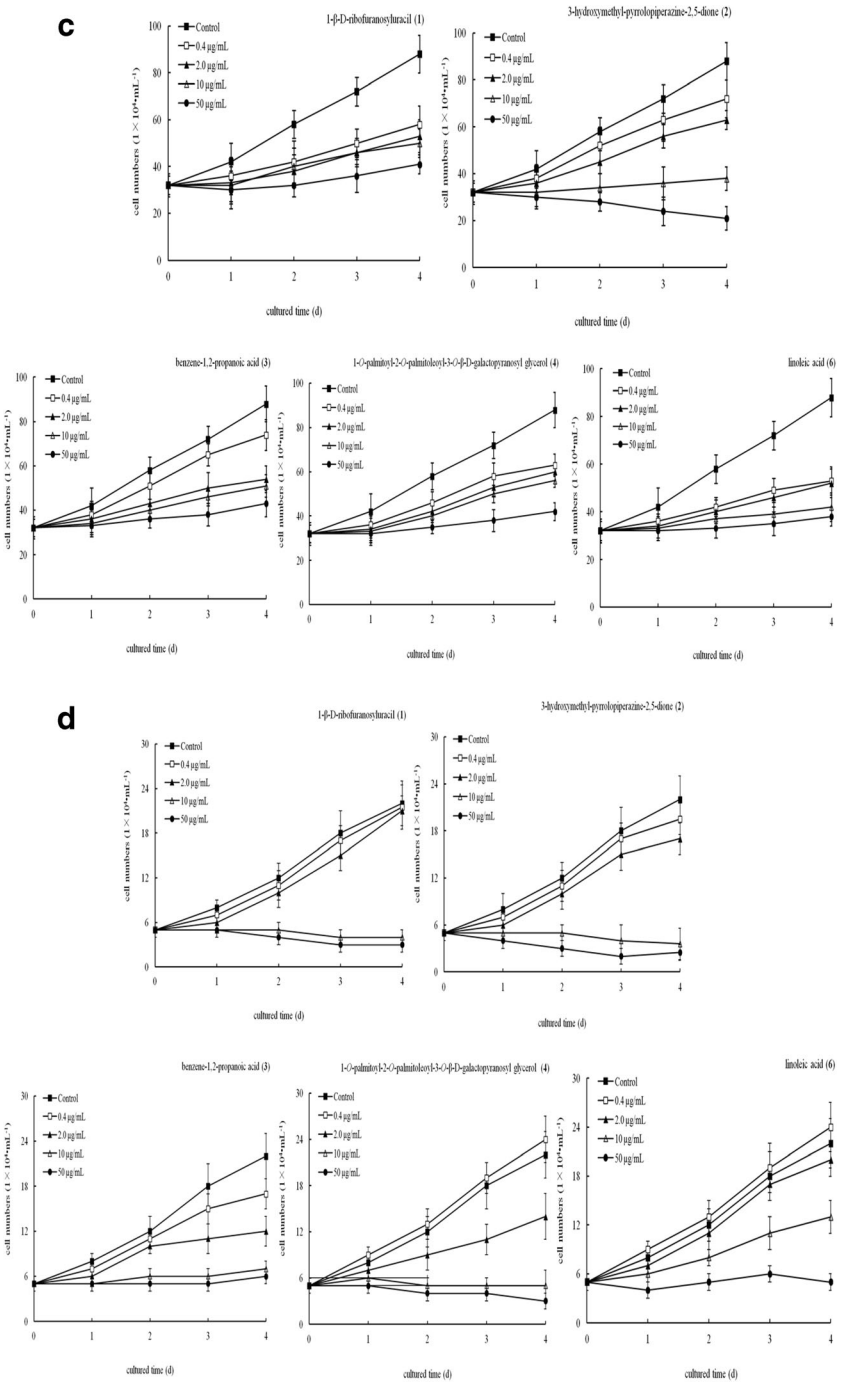

Fig. 5 (continued)

extracts of *Lithophyllum* spp. exhibited significant antialgal activities against *Heterosigma akashiwo*. Jeong et al. (2000) investigated extracts of 10 red seaweeds from the coast of Korea on the growth of *Cochlodinium polykrikoides* and found that methanol extracts of *Corallina pilulifera*, *Gigartina intermedia*, *Grateloupia prolongata* and *Porphyra yezoensis* showed algicidal activities at the concentration of 200 μg/mL. Inhibition of *Heterosigma akashiwo* that resulted from methanol extracts of 9 species of red macroalgae *Asparagopsis taxiformis*, *Bangia atropurpurea*, *Demonema pulvinatum*, *Gelidium japonicum*, *Gracilaria chorda*, *Gracilaria gigas*, *Gracilaria vermiculophylla*, *Hypnea pannosa*, and *Symphyocladia marchantioides* was also found (Alamsjah et al. 2005). Xu et al. (2005a) screened methanol extracts of 10 red macroalgae, such as *Ahnfeltiopsis flabelliformis*, *Chondrus ocellatus*, *Gelidium amansii*,

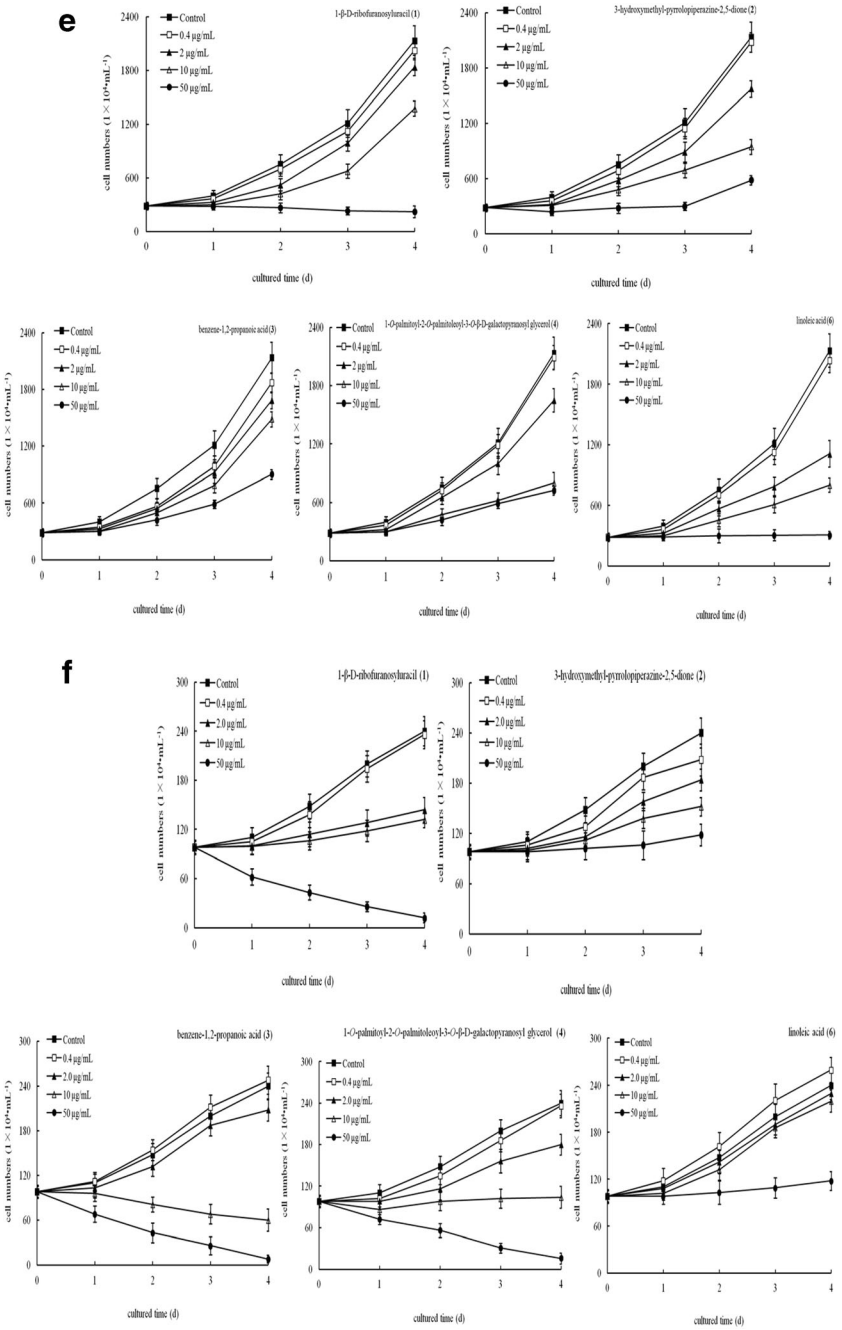

Fig. 5 (continued)

Gracilaria bursa-pastoris, *Grateloupia filicina*, *Gymnogongrus flabelliformis*, *Halymenia floresia*, *Plocamium telfairiae*, *Polysiphonia japonia*, and *Symphyocladia latiuscula*, and pointed out all 9 red macroalgae inhibited the growth of *Heterosigma akashiwo* besides *Chondrus ocellatus*. Several red macroalgae, such as *Gracilaria lemaneiformis* (Xu et al. 2005b), *Chondracanthus intermedius* (Xu 2008), *Symphyocladia marchantioides* (Xu 2008), *Porphyra tenera* (An et al. 2008), *Lithophyllum yessoense* (Luyen et al. 2009), *Chondria crassicaulis* (Jin 2011), and *Grateloupia ramosiss* (Jin 2011), have been found to inhibit some red tide microalgae, *Alexandrium tamarense*, *Heterosigma akashiwo* and *Skeletonema costatum*. Recently, the potential of red seaweeds as a source of active compounds against red tide microalgae has been confirmed in different studies (Hirao et al. 2012; Li et al. 2012; Lu et al. 2011a, b; Sun et al. 2016, 2017, 2018a, b; Tang et al. 2015; Ye and Zhang 2013).

Previous work showed that methanol extracts of *Gracilaria lemaneiformis* dry powder inhibited *Amphidinium hoefleri* and *Alexandrium tamarense* at the concentration of 2000 μg/mL; at a concentration of 1000 μg/mL, methanol extracts

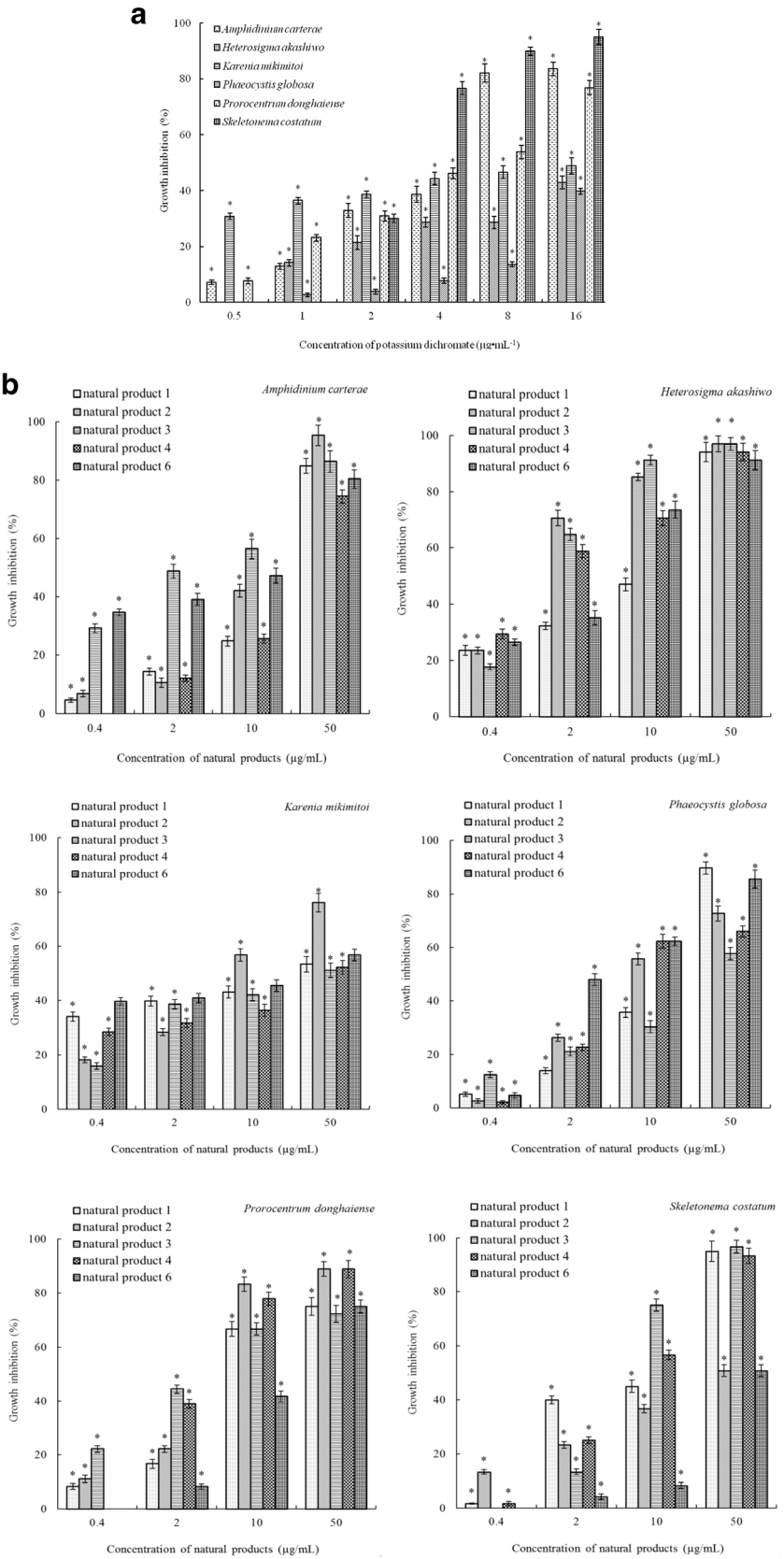

Fig. 6 (**a**) Growth inhibition of potassium dichromate against the growth of six species of red tide microalgae. (**b**) Growth inhibition of natural products (**1**, **2**, **3**, **4**, and **6**) against the growth of six species of red tide microalgae.

Table 7 $EC_{50–96\ h}$ values (μg/mL) of potassium dichromate and five natural products against several red tide microalgae

	Potassium dichromate	Natural products				
		1	**2**	**3**	**4**	**6**
A. carterae	3.9	14	9.2	2.7	20	23
H. akashiwo	36	16	1.2	1.5	1.6	2.6
K. mikimotoi	16	28	9.1	37	6.2	5.3
P. globosa	38	8.7	4.1	4.1	3.0	16
P. donghaiense	5.0	32	7.0	86	–	160
S. costatum	2.7	6.0	45	6.0	7.0	72

"–" no calculated

exhibited strongest antialgal active against *Karenia mikimotoi* (Sun et al. 2011). In this study, ethanol extracts of *Gracilaria lemaneiformis* also have significant inhibitory activities against *Karenia mikimotoi* and *Skeletonema costatum* at the concentration of 800 μg/mL (Fig. 3). The growth of red tide microalga *Cochlodinium polykrikoides* was significantly restrained by methanol extracts of four red seaweeds (*Corallina pilulifera*, *Gigartina intermedia*, *Grateloupia prolongata*, and *Porphyra yezoensis*) at a concentration of 200 μg/mL (Jeong et al. 2000). All these results showed that the intensity of inhibitory activities of red seaweeds extracts is related not only to algae species but also to red tide microalgae species. The remediation from red macroalgae of Gracilariales in eutrophic water bodies has been reported in China (Tang et al. 2003; Xu et al. 2004; Yu et al. 2017). Our term has pointed that ten species of Gracilariales showed growth inhibition to some red tide microalgae up to now (Sun et al. 2019), such as *Gracilaria asiatica* (Wang et al. 2012a, b), *Gracilaria bursa-pastoris* (Xu et al. 2005a, b), *Gracilaria chorda* (Alamsjah et al. 2005), *Gracilaria chouae* (Alamsjah et al. 2005), *Gracilaria gigas* (Alamsjah et al. 2005), *Gracilaria lemaneiformis* (Lu 2011; Sun et al. 2017; Xu et al. 2005a, b), *Gracilaria tenuistipitata* (Ye and Zhang 2013), *Gracilaria tenuistipitata* var. liui (Tang et al. 2003), *Gracilaria vermiculophylla* (Alamsjah et al. 2005), and *Gracilaria verrucosa* (Tanabe et al. 1993). Hence, they have drawn great attention. However, there was little information about isolation and identification of antialgal compounds in red macroalgae of Gracilariales. Except *Gracilaria lemaneiformis* (Lu 2011; Sun et al. 2017), antialgal compounds in other red macroalgae of Gracilariales not have been reported. However, in terms of antialgal compounds in *Gracilaria lemaneiformis*, their research was far away from enough.

Several researchers have isolated and/or identified some chemical constituents from *Gracilaria lemaneiformis* (Chen et al. 2004; Lu 2011; Lu et al. 2009, 2011a, b; Mei et al. 2006; Sun et al. 2017, 2018a; Zhang et al. 2012), such as 8-hydroxy-4E,6E-octadien-3-one (Lu 2011), 1,3-dipalmitin (Lu et al. 2011a), linoleic acid (Lu et al. 2011b), and oleic acid (Chen et al. 2004; Lu et al. 2009; Mei et al. 2006; Zhang et al. 2012; Zhang 2011). In order to clearly reflect the differences in results between these reported literatures and our this work, the isolation (or analysis) process and identified compounds are summarized in Table 8. In our current study, we have purified from ethanol extracts of *Gracilaria lemaneiformis* 1-β-D-ribofuranosyluracil (**1**), 3-hydroxymethyl-pyrrolopiperazine-2,5-dione (**2**), benzene-1,2-propanoic acid (**3**), 7-oxabicyclo[4.1.0]-heptan-3-ol (**5**), 3,4-dimethoxy-6-(methoxymethyl)-tetrahydro-2H-pyran-2,5-diol (**7**) and 3,7,11,16- tetramethyl -2-heptadecen-1-ol (**8**) besides benzene-1,2-propanoic acid (**3**), 1-*O*-palmitoyl-2-*O*-palmitoleoyl-3-*O*-β-D-galactopyranosyl glycerol (**4**) and linoleic acid (**6**) that previously isolated from *Gracilaria lemaneiformis* (Lu 2011; Lu et al. 2011a; Sun et al. 2018a) (Fig. 4). It could be seen very clearly that five natural products **1**, **2**, **5**, **7** and **8** were isolated from this marine macroalgae for the first time. Further, after searching the metabolites from marine macroalgae, we found that natural products **3**, **5**, and **8** were isolated from marine macroalgae for the first time. There were at least 80 chemical constituents in *Gracilaria lemaneiformis* (Chen et al. 2004; Lu 2011; Lu et al. 2009, 2011a, b; Mei et al. 2006; Zhang et al. 2012), but no more than 30 chemical components could be isolated and purified (Table 8). And among these components which isolated and purified from *Gracilaria lemaneiformis*, the components with antialgal activity were less than 20 (Lu 2011; Lu et al. 2011b; Sun et al. 2017).

Antialgal activity evaluation showed that five natural products (**1**, **2**, **3**, **4**, and **6**) have selective antialgal activities against tested six red tide microalgae (Figs. 5a–f, 6b, and 12; Table 7). Further, we compared the $EC_{50–96\ h}$ values of five natural products obtained in this work and all natural products reported, such as gossonorol isolated from *Porphyra yezoensis* (Sun et al. 2018b), trehalose (Sun et al. 2018b), 1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester (Kang 2006) purified from *Ulva pertusa*, and 8-hydroxy-4E,6E-octadien-3-on obtained from *Gracilaria lemaneiformis* (Lu 2011; Sun et al. 2017), and found that natural product **3** (natural product **4**) showed the superior application potential than potassium dichromate and other reported natural products as a characteristic antialgal agent against *Amphidinium carterae* (*Phaeocystis globosa*).

In conclusion, fifteen natural products (8 in this work (Fig. 4), 7 in our previous study (Sun et al. 2017)) with antialgal activities were obtained from *Gracilaria lemaneiformis*, and they exhibit a range of antialgal activities including inhibition effects on *Amphidinium carterae*, *Heterosigma akashiwo*, *Karenia mikimotoi*, *Phaeocystis globosa*, *Prorocentrum donghaiense*, and *Skeletonema costatum*. Their inhibitory activities were evaluated and it was found that five natural

Table 8 The isolation process and identified compounds from *Gracilaria lemaneiformis*

Extracts	Isolation and purification	Identified compounds	Reference
Mixture of methanol, acetone and distilled water extraction (7:7:6)	Silica gel column chromatography and repeated preparative thin-layer chromatography	Benzene-1,2-propanoic acid, gossonorol, 7,10-epoxy-ar-bisabol-11-ol, and p-hydroxyphenyl ethanol	Sun et al. (2018c)
Mixture of methanol, acetone and distilled water extraction (7:7:6)	Silica gel column chromatography and repeated preparative thin-layer chromatography	Gossonorol, 7,10-epoxy-ar-bisabol-11-ol, glycerol monopalmitate, stigmasterol, 15-hydroxymethyl-2,6,10,18,22,26,30-heptamethyl-14-methylene-17-hentriacontane, 4-hydroxyphenethyl alcohol, and margaric acid	Sun et al. (2017)
Ethanol extraction	Pressure and reverse silica gel column chromatography, Sephadex LH-20 gel column chromatography, and HPLC	Palmitic acid, linoleic acid, 1-*O*-palmitoyl-3-*O*-β-D-galactopyranosyl glycerol, squamolone, 2-ethylidene-3-methylsuccinimide,1-*O*-palmitoyl-2-*O*-oleoyl-3-*O*-β-D-galactopyranosyl glycerol, 1-*O*-palmitoyl-2-*O*-palmitoleoyl-3-*O*-β-D-galactopyranosyl glycerol, *N*-phenethulacetamide, butylisobutyl phthalate, 1,4-dibenzoyloxybenzene, loniolide, 8-hydroxy-4E,6E-octadien-3-one, 3β-hydroxy-5α,6α-epoxy-7-megastigmen-9-one, adenosine, and uridine	Lu (2011); Lu et al. (2011b)
80% ethanol extraction	Normal phase silica gel and Sephadex LH-20 gel column chromatography	Palmitic acid, 1,3-dipalmitin, 1-monopalmitin, uracil, adenosine, 2α-oxa-2-oxo-5α-hydroxy-3,4-dinorcholestane, 1-*O*-β-D-galactosyl-(6→1)-α-D-galactosyl-2,3-*O*-dihexadecanoyl-glycerol, 1-*O*-α-D-glucose-2,3-*O*-dihexadecanoyl-glycerol, and β-sitosterol	Lu et al. (2011a)
Mixture of chloroform, methanol and distilled water extraction (1:2:0.8)	This extract did not continue to isolation and purification, gas chromatography-mass spectrometry was carried out	C16 and C18 fatty acids	Zhang et al. (2012)
Headspace solid-phase micro--extraction coupled	The extract did not continue to isolation and purification, GC-MS analysis was conducted	Volatile organic compounds, benzylideneacetone, and 6,10,14-trimethyl-2-pentadecanone	Zhang (2011)
Ethanol extraction	The extract was extracted with ethyl ether, and did not continue to isolation and purification, GC-MS analysis was conducted	Palmitic acid, stearic acid, palmitoleic acid, olei cacid, and linoleic acid	Mei et al. (2006)
Hot water extraction	This extract was loaded on DEAE-Sephadex A-25 anion exchange chromatograph	Three single polysaccharide components	Lu et al. (2009)
Distilled water extraction	After precipitation with ammonium sulfate, extract was further separated through Sephadex G-100 column	Phycobiliproteins	Chen et al. (2004)

products (**1**, **2**, **3**, **4**, and **6**) purified from this study showed good superiority than these seven natural products isolated from our previous research in inhibiting *Amphidinium carterae*; growth inhibition of these natural products against *Heterosigma akashiwo*, *Karenia mikimotoi*, *Prorocentrum donghaiense*, and *Skeletonema costatum* was very close; for *Phaeocystis globosa*, seven natural products except glycerol monopalmitate isolated in our previous research (Sun et al. 2017) have stronger inhibition effects than these five natural products purified in this study. These results give a conclusion that *Gracilaria lemaneiformis* was a new source of natural products with antialgal activities.

Conclusions

We now report the purification of the eight natural products and a study of their antialgal effects on the growth of the red tide microalgae, *Amphidinium carterae*, *Heterosigma akashiwo*, *Karenia mikimotoi*, *Phaeocystis globosa*, *Prorocentrum donghaiense*, and *Skeletonema costatum*. And it clearly pointed out that two compounds, benzene-1,2-propanoic acid (**3**) and 1-*O*-palmitoyl-2-*O*-palmitoleoyl-3-*O*-β-D-galactopyranosyl glycerol (**4**), are expected to be developed into environment-friendly antialgal agent against *Amphidinium carterae* or *Phaeocystis globosa*.

Recently, the isolation of natural products with antialgal activities from marine macroalgae has been regarded as an environmentally friendly alternative approach for controlling red tide microalgae in marine systems (Accoroni et al. 2015; Alamsjah et al. 2005; An et al. 2008; Ben Gharbia et al. 2017; Jeong et al. 2000; Nan et al. 2004; Sun et al. 2015; Tang and Gobler 2011). These natural products include a variety of bioactive molecules such as monoterpenes (Konig et al. 1999), bromide (Ohsawa et al. 2001), polyphenol (Gross 2003), phenylpropanoids (Sun et al. 2018a), miscellaneous compounds (Macías et al. 2008; Sun et al. 2016, 2017, 2018a, b, c), and other not yet identified compounds (Accoroni et al. 2015; An et al. 2008; Ben Gharbia et al. 2017; Chowdhury et al. 2014; Sun et al. 2016; Tang and Gobler 2011; Wang et al. 2007), and *etc*. However, this is far from enough for biological control of red tide microalgae using natural products with antialgal activities isolated from marine macroalgae. Therefore, it is urgent for researchers to screen and isolate antialgal compounds from more species of marine macroalgae.

Authors' contributions Ying-ying Sun performed the data analyses and wrote the manuscript; Jing Zhou contributed significantly to analysis and manuscript preparation; Xiu Han, Zi-xuan Yang, and Xin Zhang performed the experiment; Nai-sheng Zhang helped perform the analysis with constructive discussions.

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Data availability The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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Consent to publish Written informed consent for publication was obtained from all participants.

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